

THE CONSTITUENTS
OF
TARAXACUM ROOT

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(*From the Transactions of the Chemical Society, Vol. 101, 1912*)



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CCLII.—*The Constituents of Taraxacum Root.*

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THE root of the common dandelion (*Taraxacum officinale*, Wiggers) appears to have been employed medicinally for several centuries, and it still maintains a place in the more important national Pharmacopœias. It is therefore somewhat remarkable that up to the present time so little of a definite nature should be known respecting its constituents, for, apart from the observed presence of inulin—which is common to the family of *Compositæ*—lævulin, and such ordinary constituents of plants as sugar, resin, and mucilage, no well-characterised compound has hitherto been isolated from this root.

Polex (*Arch. Pharm.*, 1839, **19**, 50) has stated that on boiling the milky juice of taraxacum with water, filtering and concentrating the liquid, a crystalline substance was obtained which was sparingly soluble in cold water, but readily so in boiling water, alcohol, or ether, and possessed an agreeably bitter, somewhat acrid taste. This substance was termed “taraxacin,” but no analysis, melting point, or other characters were recorded which would serve for its identification. It was also noted by Polex (*loc. cit.*) that the resinous and albuminous material which separated on heating the milky juice to boiling, when extracted with alcohol, yielded a substance which crystallised in a white, cauliflower-like form.

Kromayer (*Arch. Pharm.*, 1861, **105**, 6) examined the dried milky juice of the plant, for which he proposed the name “leontodonum.” From the portion of this which was soluble in water he obtained some crystals mixed with amorphous material, but did not succeed in isolating the so-called “taraxacin.” The portion of the dried milky juice which was insoluble in water yielded, on extraction with alcohol, “tasteless, spherical granules,” which the author designated as “taraxacerin.” An analysis (C=79·44; H=12·69 per cent.) was recorded of this substance, but no melting point, and to it the formula C₄₀H₈₀O₅ (or the simpler expression C₈H₁₆O) has since been assigned.

It is apparent from present knowledge that the so-called “taraxacin” and “taraxacerin” of the above-mentioned authors could not have been pure or homogeneous substances. The statements which have subsequently been recorded in the literature respecting the proportion of “taraxacin” in taraxacum root, with the assumption that it represents a distinct bitter principle, are therefore quite illusory.

L. E. Sayre has more recently contributed a number of papers on the subject of taraxacum (*Proc. Amer. Pharm. Assoc.*, 1893, p. 77; 1894, p. 241; 1895, p. 203; 1896, p. 160; 1897, p. 223; 1898, p. 341), but his investigations do not appear to have resulted in the isolation of any definite constituent of the root.

The question regarding the occurrence of mannitol in taraxacum root was investigated many years ago by T. and H. Smith (*Pharm. J.*, 1849, **8**, 480), who conclusively proved that this compound does not pre-exist therein, but that it is formed when an extract of the root undergoes the so-called mucous or viscous fermentation. Its formation under these conditions would appear to permit of explanation by the fact that taraxacum root contains an abundance of inulin, which, on hydrolysis, is converted into lävulose, and the latter, by the special fermentative process referred to, becomes reduced to mannitol. The above observation has now also been confirmed by the present authors, inasmuch as no trace of mannitol could be isolated directly from the root employed for this research.

While the present investigation was in progress it has been recorded (*Brit. Med. J.*, May 25th, 1912, p. 1181) that the use of taraxacum in cases of cancer has been attended with remarkably beneficial results, and shortly afterwards (*ibid.*, July 13th, 1912, p. 97) attention was directed to the use of choline in the treatment of this disease. Additional interest is imparted to these two quite independent observations, especially when considered conjointly, by the fact that taraxacum root has now been found to contain choline. The various other well-defined compounds which have been isolated are summarised at the end of this paper.

EXPERIMENTAL.

The material employed for this investigation consisted of the best quality of English taraxacum root, which was collected in the autumn of 1911, and kindly supplied to us by Messrs. W. Ransom and Son, of Hitchin.

A small portion (25 grams) of the ground root was treated with Prolli's fluid, and the resulting product tested for an alkaloid with the usual reagents. The reactions obtained were so slight as to indicate the presence of not more than traces of an alkaloidal substance.

Another portion (20 grams) of the ground material was successively extracted in a Soxhlet apparatus with various solvents, and the resulting extracts dried in a water-oven until of constant weight:

| | | | |
|-------------------------------------|----------|-------------|----------------|
| Petroleum (b. p. 35—60°) extracted, | 0·28 | gram = 1·40 | per cent. |
| Ether | ,, 0·06 | ,, = 0·30 | , |
| Chloroform | ,, 0·05 | ,, = 0·25 | , |
| Ethyl acetate | ,, 0·34 | ,, = 1·70 | , |
| Alcohol | ,, 2·33 | ,, = 11·65 | , |
| Water | ,, 10·20 | ,, = 51·00 | , |
| Total | 13·26 | grams = | 66·3 per cent. |

In order to ascertain whether an enzyme were present, 200 grams of the air-dried root were extracted with cold water, and to the clear, filtered liquid about twice its volume of alcohol was added. A slight precipitate was thus produced, which, when collected and dried in a vacuum over sulphuric acid, could be reduced to a brown powder. This product, which amounted to 0·85 gram, very slowly hydrolysed amygdalin, and thus possessed some enzymic activity.

For the purpose of a complete examination of the constituents of the root, 22·9 kilograms of the dried, ground material were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, 7·3 kilograms of a viscid, dark-coloured extract were obtained.

One kilogram of the alcoholic extract, representing about 3·14 kilograms of the root, was examined for sucrose by the following method: The extract was first mixed with water to separate the resin, which was incorporated with the larger portion subsequently obtained, and designated as (B). The filtered, aqueous liquid was then treated with an excess of milk of lime, again filtered, and the alkaline filtrate saturated with carbon dioxide. This liquid, after filtration, was evaporated under diminished pressure to the consistency of a syrup, and the latter treated with successive portions of alcohol until a product was finally obtained, which dissolved completely in alcohol of about 85 per cent. strength. The solution of this product, when decolorised with a little animal charcoal and kept for several months, deposited no crystalline substance, and there was therefore no indication of the presence of sucrose.

Distillation of the Extract with Steam. Separation of an Essential Oil.

The entire remaining portion (6·3 kilograms) of the above-mentioned alcoholic extract of the root was mixed with water, and distilled in a current of steam. The distillate was extracted with ether, the ethereal liquid dried, and the solvent removed, when a small amount of a dark yellow essential oil was obtained. This oil had a strong, persistent odour, and gave the colour reaction for furfuraldehyde.

Non-volatile Constituents of the Extract.

After the above-described operation there remained in the distillation flask a dark-coloured aqueous liquid (*A*), together with a quantity of a soft, somewhat oily resin (*B*). The resinous material, the separation of which was attended with considerable difficulty, was finally washed thoroughly with warm water, and the washings added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid, after concentration under diminished pressure, was extracted many times with ether. These ethereal liquids were united, the greater portion of the solvent removed, and the residue mixed with about an equal volume of light petroleum (b. p. 35—50°), when a red oil was deposited. On decanting and concentrating the supernatant liquid, and again treating it with light petroleum, further small quantities of red oil were obtained, which were added to the first portion. The mixture of ether and light petroleum was finally evaporated, the residue dissolved in ether, and the ethereal liquid shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide. Each of these alkaline liquids was acidified, extracted with ether, and the solvent evaporated.

Isolation of p-Hydroxyphenylacetic Acid, C₆H₄(OH)·CH₂·CO₂H.

The product obtained from the above-mentioned ammonium carbonate extract was a dark yellow oil. This was treated with hot water and a little animal charcoal, and the liquid filtered, when, on cooling, a gum-like mass separated, which gradually became crystalline. After recrystallisation from benzene containing a little ethyl acetate, a very small amount (about 0.05 gram) of an acidic substance was obtained, which separated in colourless needles, melting at 144—145°. The above-mentioned red oil, which was deposited by the addition of light petroleum to the concentrated ethereal liquid, was redissolved in ether, and extracted successively with aqueous alkalis, as already described. The ammonium carbonate extract thus obtained, when acidified, yielded a gum-like product, which was esterified. The acid was then regenerated from the ester and crystallised several times from ethyl acetate, when it separated in flat needles melting at 144—146°, and amounted to about 0.4 gram. It was identical with the small portion (0.05 gram) of acid first obtained. By the subsequent extraction of both portions of the original ethereal extract with sodium carbonate and sodium hydroxide respectively, only small amounts of dark-

coloured, amorphous products were obtained, from which nothing definite could be isolated.

The above-described acid was dried at 105° and analysed:

0·0632 gave 0·1451 CO₂ and 0·0294 H₂O. C=62·6; H=5·2.

0·0818 „ 0·1894 CO₂ „ 0·0379 H₂O. C=63·1; H=5·1.

0·1009 neutralised 32·5 c.c. N/50-KOH. M.W. (monocarboxylic acid)=155.

C₈H₈O₃ requires C=63·1; H=5·3 per cent. M.W.=152.

A determination of the molecular weight of the acid by Barger's microscopic method was kindly made for us by Mr. A. J. Ewins, B.Sc., with the following result:

0·048 in 1·196 of absolute alcohol, using α -naphthol as the standard, was between 0·26 and 0·275 mol. Mean M.W.=150.

The acid was soluble in cold, and more readily in warm, water, as also in alcohol, ether, ethyl acetate, and acetone, but only slightly so in benzene or the higher boiling fractions of petroleum. Its dilute aqueous solution gave no perceptible coloration with ferric chloride. With Millon's reagent it yielded the deep red colour characteristic of the aromatic monohydroxy-acids (*Ber.*, 1879, **12**, 1452), and a trace of the substance, when heated with soda-lime, gave a distinct phenolic odour.

A consideration of the composition and characters of the above-described substance indicated it to be *p*-hydroxyphenylacetic acid, which has not previously been observed to occur as such in the vegetable kingdom. It was obtained by A. G. Perkin and Newbury (*Trans.*, 1899, **75**, 834) by the action of potassium hydroxide on genistein, and Ewins and Laidlaw (*J. Physiol.*, 1910, **41**, 78) have shown that when *p*-hydroxyphenylethylamine is administered by the mouth to an animal, it is transformed to a large extent into *p*-hydroxyphenylacetic acid, which may subsequently be extracted from the urine.

In order completely to establish the identity of the substance above described with *p*-hydroxyphenylacetic acid, it was deemed desirable to compare it with the synthetic acid, especially as it had been stated by Salkowski (*Ber.*, 1879, **12**, 1438), who first effected its synthesis, that its aqueous solution gives with ferric chloride a slight greyish-violet coloration, which immediately changes to a dirty greyish-green. It was, moreover, thought possible that the coloration given by the synthetic product might be due to a slight contamination with the corresponding ortho-compound, which is known to produce a violet colour with ferric chloride. Baumann (*Ber.*, 1880, **13**, 279), who obtained *p*-hydroxyphenylacetic acid

from human urine, has, however, also noted that its aqueous solution gives with ferric chloride a slight violet coloration.

A quantity of the synthetic acid was accordingly prepared from phenylacetic acid, the latter having first been nitrated according to the method of Maxwell (*Ber.*, 1879, **12**, 1765). After very prolonged fractional crystallisation from warm water, a product was obtained which melted at 152° , the pure *p*-nitrophenylacetic acid having been observed by Maxwell (*loc. cit.*) to melt at 150° , and by Bedson (*Trans.*, 1880, **37**, 91) at $150-151^{\circ}$. This nitro-acid was reduced by tin and hydrochloric acid, and then, by means of the diazo-compound, converted into the corresponding hydroxy-acid. The acid thus prepared was found to have the same melting point as that obtained from taraxacum root, and when the two products were mixed no depression of the melting point ensued. The reaction with Millon's reagent was precisely the same as that previously mentioned. On comparing the behaviour of the natural and the synthetic acid towards ferric chloride, it was observed in both cases that if a fairly concentrated solution of the acid were employed a faint and exceedingly fugitive violet coloration was produced, rapidly changing to greenish-brown, thus confirming the observations of Salkowski and of Baumann (*loc. cit.*).

The above results thus completely established the identity of the acid from taraxacum root with *p*-hydroxyphenylacetic acid.

After the extraction of the original aqueous liquid with ether, as above described, it was shaken with eighteen successive portions of warm amyl alcohol. These amyl-alcoholic liquids were united, washed with water, concentrated under diminished pressure to the consistency of a syrup, and the last traces of amyl alcohol removed by passing steam through the liquid. The syrupy product was then further concentrated under diminished pressure, afterwards on a water-bath, and finally dried as completely as possible in a vacuum desiccator. There was thus obtained a quantity (42·5 grams) of a dark brown, viscous mass, which possessed a strongly bitter taste, and in aqueous solution gave a dark green colour with ferric chloride.

Twelve grams of the above-mentioned product were heated for two hours with 5 per cent. sulphuric acid in aqueous alcohol. On subsequently distilling the mixture in a current of steam, a very small amount of a yellow oil was obtained, which gave the colour reaction of furfuraldehyde. The aqueous acid liquid was then extracted many times with ether, after which the sulphuric acid was removed by barium hydroxide, the excess of the latter by carbon dioxide, and the filtered liquid concentrated. From this syrupy product a small amount of an osazone (m. p. $210-211^{\circ}$) was

prepared, thus indicating that some glucosidic material was contained in the amyl-alcoholic extract. The above-mentioned ethereal extract of the aqueous acid liquid was thoroughly extracted with aqueous ammonium carbonate, after which the ethereal liquid was dried and evaporated, but only a trace of yellow, amorphous material remained. On acidifying the ammonium carbonate extract, however, extracting many times with ether, and evaporating the solvent, a small amount of a crystalline substance was deposited. After recrystallisation from ethyl acetate this was obtained in thin, flat needles, melting at 146° , and was identical with the *p*-hydroxyphenylacetic acid, $C_8H_8O_3$, previously described. (Found, C = 62.5; H = 5.4. Calc., C = 63.1; H = 5.3 per cent.) The amount thus obtained was only 0.09 gram.

Another portion (27 grams) of the above-mentioned amyl-alcoholic extract was heated for a few minutes with a 10 per cent. solution of potassium hydroxide; the mixture then rapidly cooled and acidified, when a quantity of resinous material separated. This was collected, mixed with purified sawdust, and the dried mixture thoroughly extracted with ether. The aqueous acid liquid from which the resin had been removed was likewise extracted many times with ether, after which the two ethereal liquids were united and extracted with aqueous ammonium carbonate. On subsequently evaporating the ether there remained a slight residue, from which a crystalline substance melting at $233-235^{\circ}$ was obtained. This substance was very soluble in chloroform, almost insoluble in ethyl acetate, and gave no coloration with ferric chloride, but the amount obtained (0.04 gram) was too small to permit of its further examination. The ammonium carbonate extract, when acidified and extracted with ether, yielded a small amount of a semi-crystalline product, which was readily soluble in warm water, and its solution gave with ferric chloride a deep green colour. The whole of this product was heated with chloroform containing a trace of ethyl acetate, when a small amount of a brown substance remained undissolved. The latter was crystallised from very dilute alcohol, when 0.07 gram of an acid was obtained, which melted and decomposed at 214° , and gave a deep green colour with ferric chloride. This substance was evidently 3:4-dihydroxycinnamic acid, since no depression of the melting point ensued when mixed with a pure specimen of the respective acid, and it was subsequently obtained in an amount which permitted of its complete identification, as will be further noted. The portion of the above-mentioned product which had dissolved in the mixture of chloroform and ethyl acetate formed, after the evaporation of the solvent, a viscid mass, which was repeatedly extracted with boiling benzene. From the latter liquid some crystals were deposited, which, after several crystallisa-

tions from benzene containing a little alcohol, separated in flat needles melting at 145—146°. This substance amounted to 0·25 gram, and was identified as *p*-hydroxyphenylacetic acid. (Found, C=62·6; H=5·4. Calc., C=63·1; H=5·3 per cent.)

After the extraction of the original aqueous liquid with amyl alcohol, as above described, the last traces of the latter were removed by a current of steam. The liquid was then treated with a solution of basic lead acetate until no further precipitate was produced, the precipitate collected and thoroughly washed with water, the washings being added to the main portion of the filtered liquid.

Isolation of 3:4-Dihydroxycinnamic Acid,
 $C_6H_3(OH)_2\cdot CH:CH\cdot CO_2H$.

A portion of the above-mentioned basic lead acetate precipitate, representing 2 kilograms of the original alcoholic extract, was suspended in water, decomposed by hydrogen sulphide, and the mixture filtered. The filtered liquid was then concentrated under diminished pressure to the consistency of a thin syrup. It gave a dark brown coloration with ferric chloride, but no precipitate with gelatin, thus indicating the absence of tannin, and it also gave no reaction with potassium-mercuric iodide. As nothing separated from the liquid on keeping, it was heated to boiling, neutralised with potassium hydroxide, and sufficient of a concentrated solution of the latter added to represent about 10 per cent. of the mixture, after which it was boiled for about five minutes. The liquid was then poured into dilute sulphuric acid, and, after cooling, the mixture was extracted many times with ether, the combined ethereal liquids being subsequently extracted with aqueous ammonium carbonate and sodium hydroxide. Nothing of interest was removed by the last-mentioned alkali, and on finally evaporating the ether only a trace of amorphous material remained. The ammonium carbonate extract, however, after acidification and extraction with ether, yielded a product which gave with ferric chloride a dark green colour. From this product, after several crystallisations from hot water containing a little alcohol, a small amount (about 0·2 gram) of an acid was obtained, which melted and decomposed at 214—216° with evolution of gas. It was dried at 110°, and analysed:

0·0643 gave 0·1405 CO₂ and 0·0265 H₂O. C=59·6; H=4·6.
 $C_9H_8O_4$ requires C=60·0; H=4·4 per cent.

The above-described substance was thus definitely identified as 3:4-dihydroxycinnamic acid, a smaller amount of which had previ-

ously been obtained from the amyl-alcoholic extract of the original aqueous liquid.

The filtrate from the precipitate produced by basic lead acetate was treated with hydrogen sulphide for the removal of the excess of lead, again filtered, and concentrated under diminished pressure to the consistency of a syrup. It evidently contained an abundance of sugar, since it readily yielded *d*-phenylglucosazone, melting at 204—206°. A portion of the syrup was acetylated, but as nothing crystalline separated from the product, even after long keeping, it was finally hydrolysed. The regenerated sugar was then found to be strongly laevorotatory, thus indicating that it must have consisted, to a large extent at least, of laevulose. Another portion of the syrup was heated for about two hours with 5 per cent. sulphuric acid, but, with the exception of traces of furfuraldehyde, it yielded nothing definite by this treatment. A further portion of the syrup was mixed with purified sawdust, and the dried mixture extracted successively in a Soxhlet apparatus with ether, chloroform, and ethyl acetate, but only small amounts of sugary material were thus removed.

The original syrupy liquid, when heated with an alkali hydroxide, developed a strongly basic, ammoniacal odour, and it gave an appreciable precipitate with a solution of iodine in potassium iodide.

Isolation of Choline, C₅H₁₅O₂N.

The main portion of the above-mentioned syrupy aqueous liquid was thoroughly extracted with alcohol, the resulting liquid evaporated, and the residue from the latter repeatedly treated with alcohol, in the same manner, until a product was finally obtained which was soluble in nearly absolute alcohol. By this means a large proportion of the sugar was eliminated, together with any other material which was sparingly soluble in alcohol. To the alcoholic solution thus obtained a saturated alcoholic solution of mercuric chloride was added, and the mixture kept in a closed vessel for a week. The precipitate which had then formed was collected, washed with a little alcohol, dissolved as completely as possible in warm water, and the solution filtered. The mercury was subsequently removed from this solution by hydrogen sulphide, the liquid again filtered, neutralised with sodium carbonate, then slightly acidified with hydrochloric acid, and finally evaporated to dryness, for the most part in a vacuum desiccator. The dry residue was treated with absolute alcohol, the filtered liquid evaporated, and the residue repeatedly so treated until free from inorganic salt.

A relatively small amount of a nearly colourless product was thus obtained, which deliquesced on exposure to the air, and the aqueous solution of which was precipitated by the usual alkaloidal reagents, as also by gold chloride. It possessed, in fact, all the recognised properties of choline chloride. A small portion of the substance was dissolved in a little water, and precipitated by a solution of auric chloride, the pale yellow precipitate being collected, washed with a little water, and dried at 100—105°:

0·0332 gave on ignition 0·0147 Au. Au=44·3.

$C_5H_{14}ONCl, AuCl_3$ requires Au=44·5 per cent.

Another portion of the substance was dissolved in absolute alcohol, and a solution of platinic chloride added. The resulting precipitate was collected, washed with a little alcohol, and dissolved in a small amount of water. After keeping for some time, reddish-brown plates were deposited, which melted and decomposed at 250—254°:

0·0460, when heated at 110°, lost 0·0010 H_2O . $H_2O=2\cdot2$.

0·0450 * gave on ignition 0·0143 Pt. Pt=31·8.

0·0844 * " " 0·0269 Pt. Pt=31·9.

$(C_5H_{14}ONCl)_2PtCl_4, H_2O$ requires $H_2O=2\cdot8$ per cent.

$(C_5H_{14}ONCl)_2PtCl_4$ requires Pt=31·7 per cent.

The occurrence of choline as a constituent of taraxacum root has thus been established.

Examination of the Resin (B).

The crude resinous material which had been separated from the aqueous liquid (A), as previously described, was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a large Soxhlet apparatus with various solvents. The weights of the products, as determined by drying small, aliquot portions in a water-oven, were as follows:

| | | | |
|--------------------------|-----------|-------|--------|
| Petroleum (b. p. 35—50°) | extracted | 329·6 | grams. |
| Ether | " | 19·6 | „ |
| Chloroform | " | 10·0 | „ |
| Ethyl acetate | " | 10·5 | „ |
| Alcohol | " | 40·0 | „ |
| | | — | |
| Total..... | | 409·7 | grams. |

As the above amount of resin was obtained from 7·3 kilograms of the original alcoholic extract, it is equivalent to about 1·8 per cent. of resin in the air-dried root.

* Anhydrous substance.

*Petroleum Extract of the Resin.**Identification of the Free Fatty Acids.*

After the removal of the solvent from the petroleum extract the residue was dissolved in ether, and the ethereal liquid shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide. The clear, alkaline liquids yielded, on acidification, only traces of fatty material, but both the sodium carbonate and sodium hydroxide produced, to some extent, emulsions, which were separated, and the ether removed, when a solid product was obtained. This consisted of the sodium salt of fatty acids. It was suspended in dilute sulphuric acid and warmed with chloroform, which removed about 15 grams of fatty acids. The latter were converted into their methyl esters, which were distilled three times under diminished pressure, and three fractions collected. The first two fractions consisted of methyl palmitate, melting at 27—28°. (Found, C=75·0; H=12·7. Calc., C=75·5; H=12·6 per cent.) The third fraction, which distilled at 206—208°/15 mm., was liquid and unsaturated:

0·2933 absorbed 0·3164 iodine. Iodine value=107·9.

For the further examination of this fraction it was converted into a lead salt, and the latter treated with ether. The portion insoluble in ether, when decomposed by hydrochloric acid, yielded 1·5 grams of a solid acid, which distilled between 230° and 235°/27 mm., and, after crystallisation, melted at 60—61°. It was identified as palmitic acid. (Found, C=75·0; H=12·8. Calc., C=75·0; H=12·5 per cent.) The portion of lead salt which was soluble in ether, when decomposed by hydrochloric acid, yielded 7 grams of liquid acids, which distilled between 220° and 235°/12 mm.:

0·1134 gave 0·3200 CO₂ and 0·1188 H₂O. C=76·9; H=11·6.

0·3898 absorbed 0·6111 iodine. Iodine value=156·8.

These results indicate that the liquid acids consisted essentially of a mixture of oleic and linolic acids, the latter predominating.

The ethereal liquid which had been shaken with aqueous alkalis, as above described, was subsequently evaporated, and the residue heated with an alcoholic solution of potassium hydroxide. The alcohol was then evaporated, water added, and the alkaline mixture extracted with ether, when a quantity of unsaponifiable material was removed, which will subsequently be described.

*Identification of the Combined Fatty Acids.**Isolation of Melissic Acid, C₃₀H₆₀O₂.*

During the above-mentioned extraction of the alkaline liquid with ether, a slight emulsion was formed. This was thoroughly

washed with ether, then freed from the latter, and brought on a filter. A small amount of substance was thus collected, which proved to be the potassium salt of a fatty acid. The acid was liberated, dissolved in chloroform, and crystallised from ethyl acetate, when it melted at 87·5—88·5°, and amounted to 0·1 gram:

0·0821 gave 0·2399 CO₂ and 0·0983 H₂O. C=79·7; H=13·3.

C₃₀H₆₀O₂ requires C=79·7; H=13·3 per cent.

The small remaining portion of the acid was converted into its methyl ester, which, after crystallisation from alcohol, melted at 72—73°.

The above-described acid was thus identified as melissic acid, which, so far as is known to us, has never previously been obtained directly from a plant.

The aqueous alkaline liquid from which the unsaponifiable material had been removed by extraction with ether, as above described, was acidified and again extracted with ether. This ethereal liquid was dried, the solvent removed, and the residual fatty acids converted into their methyl esters. The latter, when distilled under diminished pressure, passed over between 180° and 270°/9 mm., and amounted to about 55 grams. They were optically inactive. The esters were then hydrolysed, and the resulting product, which consisted of a mixture of saturated and unsaturated acids, was separated into liquid and solid portions by means of the lead salts.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 215° and 265°/18 mm., and amounted to about 40 grams. An analysis and a determination of the iodine value gave the following results:

0·1343 gave 0·3800 CO₂ and 0·1393 H₂O. C=77·2; H=11·5.

0·3100 absorbed 0·4448 iodine. Iodine value=143·5.

C₁₈H₃₄O₂ requires C=76·6; H=12·1 per cent. Iodine value=90·1.

C₁₈H₃₂O₂ , C=77·1; H=11·4 , " , " , =181·4.

C₁₈H₃₀O₂ , C=77·7; H=10·8 , " , " , =274·1.

The above results would indicate that the liquid acids consisted chiefly of a mixture of oleic and linolic acids, with possibly a little linolenic acid.

The Solid Acids.—These acids, which amounted to 10 grams, were fractionally crystallised from ethyl acetate. The least soluble fraction so obtained separated in small needles, which melted quite constantly at 82—84°:

0·0673 gave 0·1946 CO₂ and 0·0820 H₂O. C=78·8; H=13·5.

C₂₇H₅₄O₂ requires C=79·0; H=13·2 per cent.

This acid was thus identified as cerotic acid, although the melting point was somewhat higher than that usually assigned to it.

The next two fractions, of lower melting point (75—77°), also appeared to consist essentially of cerotic acid, since they gave on analysis the following figures: C=78·5; H=13·2 per cent.

The most readily soluble fractions, which melted at 56—58°, were distilled under diminished pressure, when practically the whole passed over between 205° and 207°/12 mm. After one crystallisation of the product it melted at 60—61°, and was identified as palmitic acid. (Found, C=75·0; H=12·7. Calc., C=75·0; H=12·5 per cent.)

Unsaponifiable Constituents of the Petroleum Extract.

The ethereal liquid, obtained by extracting the hydrolysed petroleum extract of the resin with ether, as above described, was dried, and the solvent removed, when 125 grams of a yellow solid were obtained. An attempt was first made to separate the constituents of this material by direct fractional crystallisation, but, as this was unsuccessful, the various fractions were separately acetylated, and the resulting products subjected to prolonged fractional crystallisation. The solvents employed for this purpose were ethyl acetate and a mixture of the latter with alcohol.

Isolation of a New Monohydric Alcohol, Taraxasterol, C₂₉H₄₇·OH.

After many crystallisations of the above-mentioned acetylated products, a small fraction (5·2 grams) was obtained, which separated in handsome, colourless, hexagonal plates, melting at 251—252°, and this melting point was not changed by further crystallisation. A portion of this acetyl derivative was hydrolysed by boiling it for three or four hours with an alcoholic solution of potassium hydroxide, after which the alcohol was for the most part removed, water added, and the resulting solid collected. On crystallisation from alcohol, it separated in long, colourless needles, melting at 221—222°:

0·1999, when heated at 125°, lost 0·0198 H₂O. H₂O=9·9.

0·0848 * gave 0·2625 CO₂ and 0·0902 H₂O. C=84·4; H=11·8.

0·0737 * „ 0·2284 CO₂ „ 0·0772 H₂O. C=84·5; H=11·6.

C₂₉H₄₈O, 2½H₂O requires H₂O=9·8 per cent.

C₂₉H₄₈O requires C=84·5; H=11·6 „ „

It is evident from these results that the above-described compound possesses the formula C₂₉H₄₈O, and, being a new alcohol, having properties similar to those of the phytosterols, it is proposed to

* Dried at 120°.

designate it *taraxasterol*, with reference to the source from which it has been obtained.

A determination of its optical rotatory power gave the following result:

0·4343,* made up to 20 c.c. with chloroform, gave $\alpha_D + 4^\circ 11'$ in a 2-dcm. tube, whence $[\alpha]_D + 96\cdot 3^\circ$.

Taraxasterol is homologous with two monohydric alcohols previously isolated in these laboratories from the rhizome of *Apocynum androsaemifolium*, namely, androsterol, $C_{30}H_{50}O$, and homo-androsterol, $C_{27}H_{44}O$ (Trans., 1909, 95, 739), and it gives a colour reaction similar to that yielded by the last-mentioned compounds; thus, if a small amount of the substance be dissolved in chloroform with a little acetic anhydride, and a few drops of concentrated sulphuric acid subsequently added, a pink colour is produced, which slowly changes to a dark magenta with a green fluorescence, and this colour persists for several hours. The above-mentioned alcohols, together with a homologue of taraxasterol to be subsequently described, $C_{25}H_{40}O$, constitute four members of a group which is represented by the general formula $C_nH_{2n-10}O$.

Acetyl taraxasterol, $C_{29}H_{47}O \cdot CO \cdot CH_3$.—This compound (m. p. 251—252°), the preparation and characters of which have already been described, was dried at 120° and analysed:

0·0866 gave 0·2602 CO_2 and 0·0854 H_2O . C=81·9; H=10·9.

0·0824 „ 0·2472 CO_2 „ 0·0810 H_2O . C=81·8; H=10·9.

$C_{31}H_{50}O_2$ requires C=81·9; H=11·0 per cent.

A determination of its optical rotatory power gave the following result:

0·2046,* made up to 20 c.c. with chloroform, gave $\alpha_D + 2^\circ 30'$ in a 2-dcm. tube, whence $[\alpha]_D + 122\cdot 2^\circ$.

Monobromoacetyl taraxasterol, $C_{29}H_{46}BrO \cdot CO \cdot CH_3$.—Half a gram of the above-described acetyl derivative was dissolved in chloroform, and to the cold solution a slight excess of a solution of bromine in the same solvent was slowly added. The product was crystallised from ethyl acetate, when it separated in small, colourless needles, melting at 233—234°:

0·1204 gave 0·0421 AgBr. Br=14·9.

$C_{31}H_{49}O_2Br$ requires Br=15·0 per cent.

Benzoyl taraxasterol, $C_{29}H_{47}O \cdot CO \cdot C_6H_5$.—This derivative was prepared by heating the respective alcohol for a short time with benzoyl chloride and a few drops of pyridine. The product, after several crystallisations from a mixture of alcohol and chloroform, separated in glistening leaflets melting at 232°:

* Dried at 120°.

0·0810 * gave 0·2471 CO₂ and 0·0728 H₂O. C=83·2; H=10·0.
 $C_{36}H_{52}O_2$ requires C=83·7; H=10·1 per cent.

Other well-crystallised fractions obtained from the original acetylated product above described possessed the following characters:

- I. M. p. 216—222°; $[\alpha]_D +68\cdot1^\circ$; C=81·7; H=10·9 per cent.
- II. M. p. 225—227°; $[\alpha]_D +62\cdot2^\circ$; C=81·5; H=11·1 „ „
- III. M. p. 225—235°; $[\alpha]_D +77\cdot8^\circ$; C=81·6; H=11·3 „ „

The composition and characters of these fractions indicated them to contain a substance analogous to taraxasterol, but having a lower melting point and a lower optical rotation. The mother-liquors from these fractions were evaporated, and the residues brominated. By the fractional crystallisation of the product, a further amount of the above-described monobromoacetyl taraxasterol was obtained.

Fractions of the acetylated product melting lower than those above mentioned could only be crystallised with difficulty. The mother liquors from these fractions were evaporated to dryness, the residues hydrolysed, and then treated with phthalic anhydride, both in the dry state and with the admixture of a little pyridine or xylene. No acid phthalic ester could, however, be isolated by this treatment.

*Isolation of a New Monohydric Alcohol, Homotaraxasterol,
 $C_{25}H_{39}\cdot OH$.*

The above-mentioned difficultly crystallisable fractions of acetylated material were united, hydrolysed, and the product distilled under diminished pressure, when practically the whole passed over between 335° and 340°/25 mm. The distillate, which was contaminated with some oily material, was purified by dissolving it in petroleum of high boiling point, and treatment with animal charcoal. A product was thus obtained, which, after several crystallisations from dilute alcohol, separated in small needles melting constantly at 163—164°. The substance did not undergo any appreciable loss in weight on drying at 120°, and the total amount obtained was 0·45 gram:

0·0741 gave 0·2280 CO₂ and 0·0770 H₂O. C=83·9; H=11·5.
 $C_{25}H_{40}O$ requires C=84·3; H=11·2 per cent.

The composition of this substance clearly indicated it to be a lower homologue of the above-described taraxasterol, and it yielded precisely the same colour reaction as the latter. Being also a new compound it is proposed to designate it *homotaraxasterol*.

* Dried at 120°.

A determination of its optical rotatory power gave the following result:

0·0989, made up to 20 c.c. with chloroform, gave $\alpha_D + 0^\circ 15'$ in a 2-dcm. tube, whence $[\alpha]_D + 25\cdot 3^\circ$.

Acetylhomotaraxasterol, $C_{25}H_{39}O \cdot CO \cdot CH_3$.—This compound was prepared by heating the respective alcohol with acetic anhydride. It separated from a mixture of ethyl acetate and alcohol in small, colourless needles, melting at 219—220°:

0·0654 gave 0·1943 CO₂ and 0·0645 H₂O. C=81·0; H=10·9.

$C_{27}H_{42}O_2$ requires C=81·4; H=10·5 per cent.

0·0888, made up to 20 c.c. with chloroform, gave $\alpha_D + 0^\circ 15'$ in a 2-dcm. tube, whence $[\alpha]_D + 28\cdot 1^\circ$.

A very small portion of homotaraxasterol was converted into its *benzoyl* derivative, which separated from a mixture of chloroform and alcohol in small, flat needles, melting at 202°. The amount of this compound was not sufficient for analysis.

Ether Extract of the Resin.

This extract was considerably concentrated in volume and kept for some time, when a small amount of a sparingly soluble grey substance was deposited. This was collected, and the clear, ethereal liquid then extracted successively with aqueous ammonium carbonate and sodium carbonate, which, however, removed only traces of brown, amorphous material. The ethereal liquid was finally extracted with aqueous potassium hydroxide, and the alkaline liquid acidified and extracted with ether, which removed some amorphous material, and at the same time an emulsion was formed. This was separated, washed with a little ether, and the latter removed by a current of air, when, on filtration, a further small amount of the above-mentioned grey substance was obtained. The ethereal liquid which had been extracted with alkalis, when dried and evaporated, also yielded a little of the same sparingly soluble grey substance.

Isolation of Cluytianol, $C_{29}H_{46}O(OH)_4$.

The above-described grey substance was first subjected to prolonged extraction with absolute alcohol in a Soxhlet apparatus. During this operation it was partly deposited in a nearly white condition, and, on finally concentrating the alcoholic liquid, practically all the substance separated. The material thus obtained amounted to 4·1 grams. It was subsequently heated with acetic anhydride, and the resulting product fractionally crystallised many

times from alcohol, when an acetyl derivative was obtained, which separated in colourless, flat needles, melting at 161°.

A portion of the acetyl derivative was hydrolysed by boiling with an alcoholic solution of potassium hydroxide. The product, after crystallisation from dilute pyridine, separated in minute, colourless crystals, melting and decomposing at 297°:

0·0826 gave 0·2197 CO₂ and 0·0790 H₂O. C=72·5; H=10·6.

C₂₉H₅₀O₅ requires C=72·8; H=10·5 per cent.

Although this substance agrees in its empirical composition with ipuranol, C₂₉H₄₇O₂(OH)₃, a trihydric alcohol which has been obtained in these laboratories from many sources, and also yields the same colour reaction as ipuranol, the analysis and characters of its derivatives proved it to be identical with a new tetrahydric alcohol, C₂₉H₄₆O(OH)₄, recently isolated by Tutin and Clewer from the South African plant *Cluytia similis*, Muell. Arg., and designated by them cluytianol (this vol., p. 2230).

Tetra-acetylcluytianol, C₂₉H₄₆O₅(CO·CH₃)₄.—This compound (m. p. 161°) was prepared as above described:

0·0820 gave 0·2064 CO₂ and 0·0673 H₂O. C=68·6; H=9·1.

Its molecular weight was determined by Mr. H. W. B. Clewer:

0·4326, in 26·45 of benzene, gave Δt=0·12°. M.W.=668.

C₃₇H₅₈O₉ requires C=68·7; H=9·0 per cent. M.W.=646.

A determination of its optical rotatory power gave the following result:

0·1976, made up to 20 c.c. with ethyl acetate, gave α_D=-0°24' in a 2-dcm. tube, whence [α]_D=-20·2°.

Tetrabenzoylcluytianol, C₂₉H₄₆O₅(CO·C₆H₅)₄.—A little of this compound was prepared by treating the respective alcohol with benzoyl chloride in the presence of pyridine. The product, after repeated crystallisation from a mixture of chloroform and alcohol, separated in small, colourless needles, melting at 196°:

0·0620 gave 0·1733 CO₂ and 0·0443 H₂O. C=76·2; H=7·9.

C₅₇H₆₆O₉ requires C=76·5; H=7·4 per cent.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

These extracts were dark brown, amorphous products, and amounted to 10, 10·5, and 40 grams respectively. They were separately examined, but nothing definite could be isolated from them. The ethyl acetate and alcohol extracts were therefore heated with dilute sulphuric acid in aqueous alcohol, and the mixture distilled in a current of steam. The distillate contained traces of an oily substance, which gave the colour reaction of furfuraldehyde, but no sugar appeared to be produced, and the extracts were evidently not glucosidic.

Summary and Conclusions.

The material employed for this investigation consisted of the air-dried, fresh roots of taraxacum (*Taraxacum officinale*, Wiggers), collected in the autumn from plants grown in England.

The roots were found to contain a very small amount of an enzyme, which slowly hydrolysed amygdalin.

An alcoholic extract of the root, when distilled in a current of steam, yielded a small amount of a yellow essential oil. From the portion of the extract which was soluble in water, the following substances were isolated: (i) *p*-hydroxyphenylacetic acid, $C_8H_8O_3$ (m. p. 144—146°); (ii) 3:4-dihydroxycinnamic acid, $C_9H_8O_4$ (m. p. 214—216°); (iii) a small amount of choline, $C_5H_{15}O_2N$, which was identified by its gold and platinum compounds. The aqueous liquid contained, furthermore, a considerable quantity of a laevorotatory sugar, which appeared to consist chiefly, if not entirely, of laevulose, and yielded an osazone, melting at 204—206°.

The portion of the alcoholic extract which was insoluble in water consisted of a soft, oily resin, which amounted to 1·8 per cent. of the weight of the root. From this material the following compounds were isolated: (i) a new monohydric alcohol, *taraxasterol*, $C_{29}H_{47}\cdot OH$ (m. p. 221—222°; $[\alpha]_D + 96\cdot 3^\circ$), which yielded an *acetyl* derivative (m. p. 251—252°; $[\alpha]_D + 122\cdot 2^\circ$), a *mono-bromoacetyl* derivative (m. p. 233—234°), and a *benzoyl* derivative (m. p. 232°); (ii) a new monohydric alcohol, *homotaraxasterol*, $C_{25}H_{39}\cdot OH$ (m. p. 163—164°; $[\alpha]_D + 25\cdot 3^\circ$), which yielded an *acetyl* derivative (m. p. 219—220°; $[\alpha]_D + 28\cdot 1^\circ$), and a *benzoyl* derivative (m. p. 202°). The above-mentioned alcohols, together with two previously isolated compounds, androsterol, $C_{30}H_{49}\cdot OH$, and homo-androsterol, $C_{27}H_{43}\cdot OH$ (Trans., 1909, 95, 739), constitute an homologous group, which is represented by the general formula $C_nH_{2n-10}O$. (iii) Cluytianol, $C_{29}H_{46}O(OH)_4$, melting at 297° (this vol., p. 2230), from which the tetra-*acetyl* and tetrabenzoyl derivatives were prepared; (iv) palmitic, cerotic, and melissic acids, together with a mixture of unsaturated acids, consisting chiefly of oleic and linolic acids, with, apparently, a little linolenic acid.

The bitter taste of taraxacum, which has hitherto been ascribed to the so-called "taraxacin," appears to be due chiefly to dark-coloured, amorphous material, and not to any distinct principle. It was found, for example, that the portion of an alcoholic extract of the root which is soluble in water, when repeatedly extracted with warm amyl alcohol, yielded a viscous product, which possessed an intensely bitter taste.

A consideration of the results of the present investigation renders it evident that the products which many years ago received the designations of "taraxacin" and "taraxacerin" were not only indefinite in character, but must have consisted of very complex mixtures. It is therefore desirable that these names should no longer be retained in the literature.

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